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Inhibition of the Release Factor-Dependent Termination Reaction on Ribosomes by DnaJ and the N-Terminal Peptide of Rhodanese

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A peptide consisting of the 17 N-terminal amino acids of native bovine rhodanese in combination with the chaperone DnaJ specifically inhibits release factor- and stop codon-dependent hydrolysis of *N*-formylmethionine from *N*(formyl)-methionyl-tRNA bound with AUG to salt-washed ribosomes. Neither the peptide nor DnaJ by itself causes this inhibition. The N-terminal peptide and DnaJ both singularly and combined do not affect the peptidyltransferase reaction per se. The total amount of rhodanese synthesized in the cell-free coupled transcription-translation system is reduced by the peptide, with concomitant accumulation of full-length enzymatically inactive rhodanese polypeptides on ribosomes. In combination with DnaJ, the N-terminal polypeptide inhibits the termination and release of full-length rhodanese peptides that have accumulated on *Escherichia coli* ribosomes during the course of uninhibited coupled transcription-translation in the cell-free system. This inhibition appears to involve release factor 2-mediated termination at the UGA termination codon in the coding sequence for rhodanese. It is suggested that the N-terminal peptide inhibits the binding of the release factor to ribosomes. These data appear to provide the first report of differential inhibition of the termination reaction on ribosomes without inhibition of the peptidyltransferase reaction and peptide elongation.

The importance of the N-terminal segment of rhodanese for the folding of the nascent protein into an enzymatically active conformation was indicated by our previous studies. Rhodanese is a 33-kDa sulfurtransferase (EC 2.8.1.1) found in the matrix of mammalian mitochondria. It has been used extensively as a test protein to study protein refolding from the denatured state (9). Wild-type rhodanese can be efficiently synthesized in a cell-free coupled transcription-translation system from *Escherichia coli*; however, much of the peptide product is accumulated as peptidyl-tRNA on ribosomes (12, 13). These full-length chains can be released and converted into enzymatically active species by the incubation of ribosomes with the five *E. coli* chaperones (DnaJ, DnaK, GrpE, GroEL, and GroES) under conditions with which the synthesis of additional peptide bonds cannot be detected (13).

A chemically synthesized peptide corresponding to the 17 N-terminal amino acids of rhodanese blocked the release-activation process (15). When the peptide was added during coupled transcription-translation, it reduced the amount of wild-type protein synthesized but affected the synthesis of a deletion mutant rhodanese lacking the first 23 amino acids of the native protein considerably less (15).

The mutant protein was synthesized even more efficiently than wild-type rhodanese in the coupled transcription-translation system, but in contrast to the in vitro-synthesized wild-type protein, the mutant rhodanese was enzymatically inactive. This latter result was surprising since the crystal structure of rhodanese indicates that the protein folds into two globular domains. The 32 N-terminal amino acids of the protein, which contain a mitochondrial-matrix targeting sequence (7), contact both domains but appear to be loosely structured and to lack well-defined points of interaction with the rest of the protein (8, 19). Studies in which denatured rhodanese was refolded into an enzymatically active state indicated that a peptide with the sequence of the 23 N-terminal amino acids interfered with the proper refolding of the enzyme both under unassisted conditions and in the presence of the chaperonins GroEL and GroES (17).

Indications that the N-terminal segments of nascent peptides on ribosomes may have a regulatory effect on translation and related processes were evident in data published for chloramphenicol-inducible gene expression and attenuation. Lovett and coworkers (5, 6) reported that ribosomes stalled after translation of the N-terminal 9- and 5-amino-acid sequences of two chloramphenicol-inducible genes, the *cat* and *cmlA* genes, respectively. They demonstrated that only synthetic peptides of these specific sequences inhibit peptidyltransferase reactions (16). These peptides inhibit fragment reactions on 50S ribosomal subunits (5) as well as puromycin and termination reactions on 70S ribosomes (18). It was concluded that these peptides bind to a specific region of 23S RNA because their binding was competitively inhibited by erythromycin. This antibiotic binds to a specific nucleotide sequence in domain V of 23S RNA. These observations indicate that the N-terminal peptides interfere with the peptidyltransferase reaction on the large ribosomal subunit.

Here we report that the N-terminal rhodanese peptide (first 17 amino acids of native bovine rhodanese) together with the chaperone DnaJ inhibits codon-directed termination by release factor (RF) of either formylmethionine bound to ribosomes as fMet-$tRNA_f$ or full-length nascent rhodanese bound as peptidyl-tRNA. In the cell-free coupled transcription-translation system, the N-terminal rhodanese peptide inhibits the synthesis of rhodanese and ricin but has little or no inhibitory effect on the synthesis of dihydrofolate reductase (DHFR; EC 1.5.1.3) or chloramphenicol acetyltransferase (CAT; EC 2.3.1.28). The results presented here for the N-terminal rhodanese peptide are compared with those published by Lovett and coworkers for the inhibitory effects of leader peptides from *cat* and *cmlA* gene products.

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MATERIALS AND METHODS

Materials. Nucleoside triphosphates and *E. coli* tRNA were purchased from Boehringer Mannheim (Indianapolis, Ind.). All amino acids, *E. coli* $tRNA_f^{Met}$, rifampin, and all other biochemicals were from Sigma (St. Louis, Mo.). [^{14}C]leucine and [^{35}S]methionine were purchased from NEN-Dupont (Bedford, Mass.). The chaperone DnaJ was purchased from Epicentre Technologies (Madison, Wis.).

Methods. The chemical synthesis and purification by high-performance liquid chromatography of each peptide used (the N-terminal peptide consisting of amino acids 1 to 17 and the tether peptide consisting of amino acids 142 to 156 of the bovine rhodanese sequence) have been described previously (15). Both peptides were essentially homogeneous.

One of the plasmids used was pSP65 containing the following coding sequences under the control of the SP6 promoter: rhodanese (13), ricin (10), DHFR (11), or CAT (11). The other plasmid used was pGEM-3Z (from Promega) into which the coding sequences for RF-1 and RF-2 were inserted between the *Eco*RI and *Hin*dIII sites under the control of the T7 promoter. We obtained pRF-2* and pRF1-4 from Warren Tate and coworkers (University of Otago, Dunedin, New Zealand).

The propagation of plasmids, isolation of SP6 RNA polymerase, preparation of the *E. coli* cell extract (S30), isolation of the ribosome fraction from S30, and preparation of f[^{35}S]Met-$tRNA_f$ were carried out as described previously (11). T7 RNA polymerase was isolated as outlined by Kudlicki et al. (15). The in vitro system for coupled transcription-translation (usually a 30-μl reaction mixture) has been described in detail elsewhere (11, 12). [^{14}C]leucine was the radioactive precursor. Rifampin was added to inhibit *E. coli* RNA polymerase. The plasmid was added in circular form; however, in the constructs used, no other protein could be synthesized from the transcribed mRNA. Incubation was for 30 min at 37°C. The amount of protein synthesized was quantitated by trichloroacetic acid precipitation. For some experiments, the reaction mixture was enlarged, and then after incubation, the reaction mixture was centrifuged in an airfuge for 40 min at 150,000 × *g* to separate the supernatant from the ribosome fraction. The latter was resuspended in a small volume of 20 mM Tris-HCl (pH 7.5)–10 mM $Mg(OAc)_2$–30 mM NH_4OAc–1 mM dithiothreitol.

The ribosome fraction (176 pmol of [^{14}C]leucine incorporated into nascent rhodanese) was incubated (30-μl reaction volume) in the absence or presence of the following additions: in vitro-synthesized RF-1 or RF-2 (150 ng each), N-terminal rhodanese peptide (to give 27 μM), purified *E. coli* chaperones (3.5 μg of GroEL, 1 μg of GroES, 2 μg of DnaK, 1 μg of DnaJ, and/or 1 μg of GrpE), or puromycin (2 mM final concentration). After 20 min of incubation at 37°C, ribosomes were again separated from the supernatant by airfuge centrifugation. The amount of rhodanese released into the supernatant after the second centrifugation and, as indicated, its enzymatic activity were determined.

The enzymatic activity of rhodanese was measured as described by Sörbo (20). This assay is based on the formation of thiocyanate from cyanide plus thiosulfate; quantitation is by A_{460} generated from a complex of ferric ions with thiocyanate.

Termination, as measured by the release of *N*-formylmethionine from ribosome-bound fMet-$tRNA_f$, was carried out under conditions similar to those described by Donly et al. (4). Salt-washed ribosomes were prepared from crude *E. coli* ribosomes by incubating them with 0.5 M NH_4Cl–20 mM Tris-HCl (pH 7.5)–10 mM $MgCl_2$–1 mM dithiothreitol on ice for 30 min and then collecting them by centrifugation (90 min at 45,000 rpm with a Ti 50 rotor and Beckman ultracentrifuge). The supernatant fraction (salt wash) was also saved and used as the source of RFs. f[^{35}S]Met-$tRNA_f$ (38 pmol) was bound to salt-washed ribosomes (198 pmol) in the presence of AUG trinucleotide (1.38 nmol) in a total volume of 100 μl containing 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 150 mM NH_4Cl. Incubation was for 30 min at 30°C. Then 5-μl aliquots from the first incubation were added to a second reaction mixture (50 μl) which contained 50 mM Tris-acetate (pH 7.2), 30 mM $Mg(OAc)_2$, 60 mM NH_4OAc, about 24 μg of protein of the salt wash fraction described above, and either UAA (2 nmol) or ethanol to give a 20% final concentration as described by Moffat et al. (19). Released f[^{35}S]methionine was extracted with ethyl acetate in 0.1 N HCl and quantitated by liquid scintillation counting with Ecolite (ICN, Irvine, Calif.). Without ethanol, the omission of UAA or the salt wash fraction resulted in little release of *N*-formylmethionine (0.17 pmol).

RESULTS

The N-terminal rhodanese peptide inhibits the synthesis of some but not all proteins. A peptide corresponding to the N terminus of native rhodanese (Fig. 1) was synthesized chemically and previously shown to interfere with chaperone-mediated folding of in vitro-synthesized rhodanese on ribosomes (15). The specificity and effect of this peptide on the synthesis of four proteins in the coupled transcription-translation system with unwashed *E. coli* ribosomes (11) were studied (Table 1). The N-terminal rhodanese peptide effectively inhibited the synthesis of not only rhodanese but also ricin; however, it had only very slight effects on the expression of two *E. coli* proteins. The synthesis of CAT was slightly but significantly stimulated, whereas the synthesis of DHFR was reduced up to about 20% by 4 μg (54 μM final concentration) of the N-terminal rhodanese peptide. The synthesis of rhodanese and that of ricin were inhibited by about 58 and 77%, respectively, by the same amount of this peptide. The tether peptide (Fig. 1) corresponding to amino acids 142 to 156 of the bovine rhodanese sequence, which forms a bridge between the two globular domains of rhodanese, was synthesized and isolated under similar conditions before being used as a control. This amino acid sequence also appears to be relatively unstructured in the crystal structure of rhodanese (19). This peptide has no effect on the cell-free synthesis of any of the four enzymes studied (Table 1).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
RHO	V	H	Q	V	L	Y	R^+	A	L	V	S	T	K^+	W	L	A	E^-
RICIN	I	F	P	K^+	Q	Y	P	I	I	N	F	T	T	A	G	A	T
CAT	E^-	K^+	K^+	I	T	G	Y	T	T	V	D^-	I	S	Q	W	H	R^+
DHFR	I	S	L	I	A	A	L	A	V	D^-	R^+	V	I	G	M	E^-	N
TETHER	S	E^-	P	S	R^+	P	E^-	P	A	I	F	K^+	A	T	L		

FIG. 1. N-terminal amino acid sequences of the test proteins and the sequence of the rhodanese tether peptide (amino acids 142 to 156 of bovine rhodanese). RHO, rhodanese.

The inhibitory effect of the N-terminal peptide on rhodanese synthesis was enhanced in the presence of the chaperone DnaJ (Fig. 2). Inhibition of about 16% was observed when the concentration of the peptide in the reaction mixture used for coupled transcription-translation was 15 μM. Some reduction in the amount of polypeptide product formed in the cell-free system was apparent when only DnaJ was added (Fig. 2 [open squares]). However, concentration-dependent inhibition by DnaJ was much stronger in the presence of small amounts of the N-terminal peptide (Fig. 2 [filled squares]). No similar effect was observed with the tether peptide in either the presence or absence of DnaJ (data not shown).

The N-terminal peptide and DnaJ do not inhibit the peptidyltransferase reaction. Inhibition of the total amount of synthesized polypeptides was associated with an increase in the percentage of rhodanese that remained associated with ribosomes (Fig. 2 [triangles]). These ribosome-bound polypeptides were primarily in the form of full-length rhodanese (Fig. 3). For this experiment, coupled transcription-translation was carried out under conditions similar to those used for Fig. 2. The reaction mixture was centrifuged, and then an aliquot from the

TABLE 1. Effect of the N-terminal peptide on the synthesis of different proteins

Addition and concn (μM)[a]	Amt of protein synthesized (pmol of [^{14}C]leucine incorporated)			
	Rhodanese	DHFR	CAT	Ricin
None	653	559	601	485
N-terminal peptide				
27	393	502	681	225
54	274	448	648	112
Tether peptide				
25	648	558	601	487
50	651	557	603	484

[a] Final concentration of peptide in the transcription-translation reaction mixture.

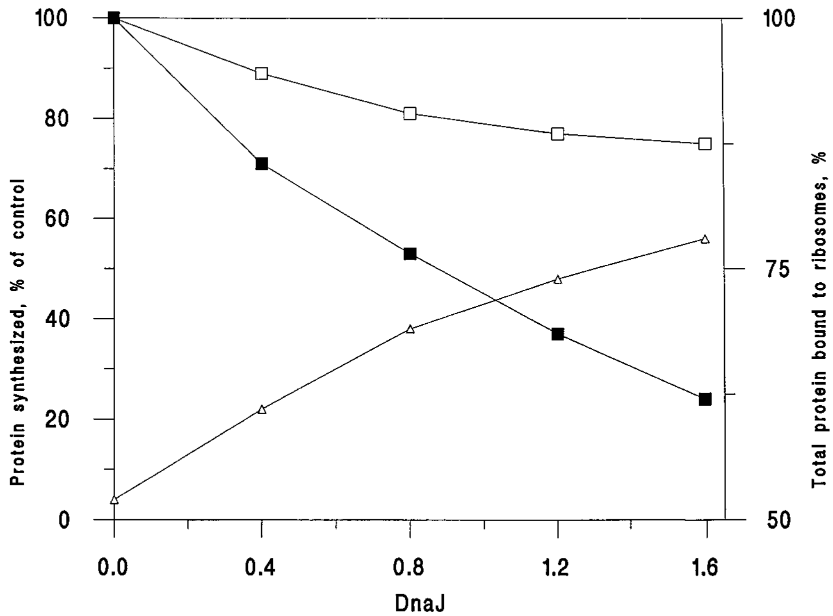


FIG. 2. Effect of DnaJ and the N-terminal rhodanese peptide on rhodanese synthesis during coupled transcription-translation. Rhodanese was synthesized by the in vitro coupled transcription-translation assay with various concentrations of DnaJ in the absence (open squares) or presence (filled squares) of 15 μM N-terminal rhodanese peptide. The abscissa represents the micromolar concentration of DnaJ in the reaction mixture. The left ordinate represents the percentage of synthesized protein as described in Materials and Methods (100% = 90 pmol of [^{14}C]leucine incorporated). Open triangles represent the percentages of total protein bound to ribosomes in the presence of different concentrations of DnaJ and 15 μM N-terminal peptide (right ordinate).

ribosome fraction was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently by autoradiography. In the uninhibited reaction, about 50% of the total synthesized polypeptides remained on ribosomes, of which about 60% was full-length rhodanese. In the presence of both the peptide and DnaJ, these numbers changed to 77 and 86%, respectively, under standard conditions, as judged by the portion of the total radioactivity applied to the gel that was recovered in the full-length rhodanese band.

The data in Fig. 2 and 3 suggest that the N-terminal peptide in concert with DnaJ inhibits the termination and release of full-length chains rather than peptide elongation. It is anticipated that direct inhibition of peptide elongation would be associated with the occurrence of a large portion of short nascent chains on ribosomes, whereas an accumulation of primarily full-length chains indicates inhibition at a termination codon. Moreover, neither the N-terminal peptide, DnaJ, nor a combination of them inhibits polyphenylalanine synthesis on ribosomes programmed with poly(U) (data not shown).

Puromycin is an antibiotic that mimics A-site-bound aminoacyl-tRNA. It reacts with peptidyl-tRNA in the ribosomal P site involving the peptidyltransferase center. If the N-terminal rhodanese peptide and DnaJ affect the peptidyltransferase reaction, the release of rhodanese peptides from ribosomes by puromycin should be inhibited. The data in Table 2 indicate that this is not the case. The results enforce our conclusion that only an RF-mediated reaction is affected.

The N-terminal peptide and DnaJ inhibit termination. In combination with DnaJ, the peptide inhibits the hydrolysis of fMet-tRNA$_f$ in the classical RF assay developed by Caskey et al. (2), as indicated by the results presented in Table 3. For these experiments, fMet-tRNA$_f$ was bound to salt-washed ribosomes with AUG and then RFs were added to the reaction mixture with either the UAA termination codon or ethanol. The release of formylmethionine in the presence or absence of the N-terminal peptide and DnaJ was measured. Inhibition

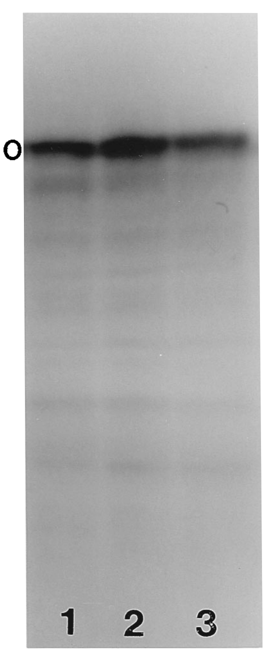
RHO


FIG. 3. Accumulation of full-length rhodanese on ribosomes during its synthesis in the presence of DnaJ and the N-terminal rhodanese peptide. Rhodanese was synthesized in vitro under different conditions by coupled transcription-translation in the presence of [^{14}C]leucine (160 Ci/mol), and then ribosomes were collected by centrifugation as described in Materials and Methods. The resuspended ribosome fractions with nascent rhodanese were analyzed by SDS-PAGE and subsequently by autoradiography. The autoradiogram is shown. Track 1, rhodanese synthesized in the absence of exogenous DnaJ and N-terminal rhodanese peptide; track 2, rhodanese synthesized in the presence of DnaJ (0.8 μM) and N-terminal peptide (15 μM); track 3, rhodanese synthesized in the presence of 30 μM N-terminal peptide only (no DnaJ added). RHO, position of full-length rhodanese.

TABLE 2. Lack of inhibition of puromycin-mediated release of nascent rhodanese by the N-terminal peptide and DnaJ

Addition(s)	Rhodanese release from ribosomes into the supernatant	
	Amt (pmol of [^{14}C]leucine)	% Release
None	26	15
Puromycin	117	69
N-terminal peptide	27	16
DnaJ	24	14
N-terminal peptide plus DnaJ	26	15
Puromycin plus N-terminal peptide	121	71
Puromycin plus DnaJ	134	79
Puromycin, N-terminal peptide, and DnaJ	141	83

was not seen with either DnaJ or the peptide alone, even at relatively high concentrations; inhibition was seen only when both were present. The RF fraction used was a crude salt wash containing the proteins removed from ribosomes with 0.5 M NH_4Cl. Adamski et al. (1) reported that large portions of RF-1 and RF-2 are bound to the ribosomes in *E. coli* cells and extracts. As originally described (2), the RF-dependent hydrolysis of fMet-tRNA$_f$ requires a termination codon, e.g., UAA for RF-1 and RF-2. In a recently published study (18), ethanol was substituted for UAA. We found that with either UAA or ethanol, only the combination of peptide and DnaJ inhibited the release of formylmethionine (Table 3). The reaction was UAA dependent in the absence of ethanol. The requirement of DnaJ and the N-terminal rhodanese peptide for the inhibition of this reaction appears to be different from those for inhibition by the N-terminal leader peptides studied by Lovett and coworkers (18). Those peptides by themselves inhibited the peptidyltransferase reaction required for RF-dependent hydrolysis of peptidyl-tRNA on the ribosome.

The N-terminal peptide affects RF-2-dependent release of rhodanese. The finding that the N-terminal peptide in combination with DnaJ efficiently inhibits RF-dependent termination and release is enforced by the results from the following experiment. Ribosomes bearing nascent rhodanese polypeptides, including full-length chains, were incubated with either RF-1 or RF-2 and with or without the N-terminal peptide, DnaJ, or all chaperones. The RF-1 or RF-2 used in these experiments was synthesized in a separate reaction from the respective plasmids by coupled transcription-translation. In vitro-synthesized RF-1 or RF-2 was partially purified by centrifugation. The supernatant fraction lacking ribosomes was used. After incubation of the samples containing ribosomes

TABLE 3. Inhibition of the termination reaction as measured by the release of fMet from fMet-tRNA$_f$ bound to ribosomes

Addition(s) or deletion	f[^{35}S]Met release (% of control)	
	UAA	Ethanol
None	100	100
Minus UAA	5	NA[a]
N-terminal peptide (27 μM)	98	109
DnaJ (0.8 μM)	93	94
N-terminal peptide + DnaJ	48	52
Tether peptide	100	100
Tether peptide + DnaJ	94	96

[a] NA, not applicable.

TABLE 4. Effects of DnaJ and the N-terminal peptide on the release process of nascent full-length rhodanese mediated by in vitro-synthesized RF-2

Addition(s)	Rhodanese release from ribosomes		Enzymatic activity	
	Amt (pmol of [^{14}C]leucine)	% Release	10^3 U	Sp act (U/mg)[a]
None	29	17	0.0	0
RF-1 only	39	23	0.48	9
RF-1 + N-terminal peptide + DnaJ	34	20	0.28	6
RF-2 only	71	42	19.0	195
RF-2 + N-terminal peptide	66	39	16.5	182
RF-2 + DnaJ	70	41	18.4	192
RF-2 + N-terminal peptide + DnaJ	37	22	0.51	10
RF-2 + all chaperones	87	51	65.6	549
RF-2 + all chaperones + N-terminal peptide	54	32	39.9	538

[a] The specific enzymatic activity of native rhodanese isolated from bovine mitochondria was 684 U of enzyme protein, as determined under the conditions used here (13).

bearing rhodanese with the additions indicated in Table 4, ribosomes were separated from the supernatant fraction by centrifugation. The supernatant fraction was analyzed for the amount of rhodanese released from ribosomes and for enzymatic activity. Release was measured as the amount of [^{14}C]leucine in the polypeptides of the supernatant fraction. Enzymatic activity was determined as described in Materials and Methods. The results are presented in Table 4. About 20% of the rhodanese polypeptides were found in the supernatant fraction after incubation with or without various combinations of RF-1, N-terminal peptide, and DnaJ. This material appears to represent polypeptides released from ribosomes by the spontaneous hydrolysis of peptidyl-tRNA or by traces of RF-1 and RF-2 present during the second incubation. RF-1 requires a UAA or UAG release codon, whereas the rhodanese coding sequence is terminated by UGA requiring RF-2. The small amount of peptide that is apparently released by RF-1 is enzymatically inactive and may be composed primarily of [^{14}C]leucine that was incorporated into trace amounts of nascent peptides initiated in intact *E. coli* cells and bound to ribosomes. An appreciable amount of the total protein synthesized in the cell-free system is released by RF-2. A large portion of this material is full-length rhodanese, as judged by SDS-PAGE (data not shown). In the absence of added chaperones, this material has low but significant levels of rhodanese enzymatic activity, which may be a result of the spontaneous folding of nascent rhodanese that occurs from the denatured state or from the small amounts of chaperones present in the reaction mixture. Relatively high levels of enzymatic activity were obtained when the five chaperones (DnaJ, DnaK, GrpE, GroEL, and GroES) were included in the reaction mixture. When corrected for short peptides (which were assumed to be enzymatically inactive), the specific enzymatic activity of this material approached that of the native enzyme. RF-2-dependent release was inhibited by the N-terminal peptide when all of the chaperones, including DnaJ, were added together with the peptide (Table 4). Rhodanese released under these conditions was enzymatically active.

DISCUSSION

The results of this study lead to the conclusion that the N-terminal peptide and DnaJ act in concert on the ribosome to block RF-dependent hydrolysis of peptidyl-tRNA immediately preceding the termination codon. Direct interaction between the N-terminal portion of rhodanese and DnaJ appears to be involved. The same conclusion was indicated from previous fluorescence studies (14) in which DnaJ caused a pronounced blue shift of the fluorescence emission spectrum obtained after specific antibodies reacted with coumarin covalently linked to the N terminus of nascent ribosome-bound rhodanese. The chaperone had no effect on this fluorescence after rhodanese had been released from the ribosome in enzymatically active form or on fluorescence from coumarin attached to the N terminus of a deletion mutant of rhodanese that lacked amino acids 1 to 23 of the wild-type enzyme. This mutant protein could not be converted into an enzymatically active form (15). Previously, we demonstrated that all five chaperones together (including DnaJ) are required during release to activate rhodanese bound to the ribosome as full-length but enzymatically inactive peptidyl-tRNA. The specific enzymatic activity of rhodanese released in the presence of the five chaperones approached that of the native enzyme (13). The N-terminal peptide inhibited this release (15). In this study, we have demonstrated that full-length rhodanese originally bound to ribosomes as peptidyl-tRNA can be released by RF-2, but this material has only about one-third of the specific enzymatic activity of the protein released in the presence of the five chaperones or of the native enzyme. Release is inhibited by the N-terminal peptide, but enzyme released in the presence of the five chaperones has high levels of specific enzymatic activity, indicating that the folding of nascent rhodanese is not directly affected by the peptide.

The most pronounced cooperative effect of DnaJ and the N-terminal peptide is seen in the assay system designed to measure the activities of RFs in terms of codon-dependent hydrolysis of fMet-$tRNA_f$ bound to ribosomes. Without each other, neither the N-terminal peptide at 28 μM nor DnaJ at 0.8 μM inhibits this reaction appreciably; however, together they cause a 50% reduction in the hydrolysis and release of *N*-formylmethionine (Table 3). These results provide direct support for the conclusion that the N-terminal peptide blocks RF-dependent termination by inhibiting the hydrolysis of peptidyl-tRNA. The data in Tables 3 and 4 indicate that the N-terminal peptide causes an accumulation of full-length rhodanese on ribosomes. This appears to be a direct result of inhibition of the RF-mediated termination reaction and hydrolysis of the peptidyl-tRNA bound at the penultimate codon preceding the stop codon (UGA in the case of rhodanese).

The results presented above provide little direct indication of the mechanism by which the N-terminal peptide might cause this inhibition. We suggest that the N-terminal segment, either as a synthetic peptide or as part of the nascent chain, binds to the site on the large ribosomal subunit to which RF-2 binds, thereby causing the inhibition of termination. In any event, the N-terminal peptide and DnaJ act as a specific inhibitory element of RF-2-mediated termination. This is in contrast to many other inhibitors of termination, such as sparsomycin, which also inhibits the release of full-length rhodanese from ribosomes (13) but which is known to be a general inhibitor of the peptidyltransferase reaction (3).

The data presented above indicate that the N-terminal rhodanese peptide acts in a different manner than do the leader peptides studied by Lovett and coworkers. They described inhibition of the peptidyltransferase reaction by the peptides themselves; both the puromycin reaction and the termination reaction were inhibited by using ribosomes bearing fMet-$tRNA_f$ (18). Interaction between these peptides and domain V of 23S RNA was indicated by competition experiments with erythromycin (5) and by direct binding studies to isolated 23S RNA (16). The data in Table 2 indicate unequivocally that the puromycin reaction, i.e., the peptidyltransferase center, is not inhibited by the N-terminal rhodanese and DnaJ. In a previous publication (13), we discussed extensively the release of nascent rhodanese polypeptides by puromycin and showed that the percentage of release is higher when it is determined directly by using ^{3}H-labeled puromycin.

It is not clear why the synthesis of rhodanese and that of ricin are inhibited by the N-terminal peptide, whereas the synthesis of DHFR or CAT is not inhibited. It may be that both rhodanese and ricin contain an embedded N-terminal signal sequence that targets transfer across membranes and/or that rhodanese and ricin require chaperone-mediated reactions that are not required for the folding of the *E. coli* enzymes CAT and DHFR. Comparisons of the N-terminal sequences (amino acids 1 to 17) of these four proteins and the tether peptide (Fig. 1) reveal no obvious similarities or dissimilarities in sequence that might account for these effects, except that there are no negatively charged amino acids in the first 16 amino acids of the N-terminal sequences of rhodanese and ricin (7).

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